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Complementarity of capture and acoustic methods for surveying Neotropical bats

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“Impossible is just a big word thrown around by small men
who find it easier to live in the world they've been given
than to explore the power they have to change it.
Impossible is not a fact. It's an opinion.
Impossible is not a declaration. It's a dare.
Impossible is potential.
Impossible is temporary.
Impossible is nothing.”

Muhammad Ali

“Though here at journey’s end
I lie in darkness buried deep,
beyond all towers strong and high,
beyond all mountains steep,
above all shadows rides the sun
and stars for ever dwell:
I will not say the day is done,
nor bid the stars farewell”

J.R.R. Tolkien

Abstract

A wide range of methods are used to sample bats, each method specialized in a particular family or group behaviour. With the increasing number of threatened bat species, there is an urgent need to increase the worldwide knowledge about this order, to improve the conservation efforts and environmental education plans. With the recent development of low-cost acoustic recorders there is an opportunity to use more standardized sampling methods turning it affordable to more projects. We tested the efficiency of this sampling strategy in one of the world's richest ecosystems, the Amazonian forest. Our study was conducted in two habitats differentiated by canopy cover, *Terra Firme* forest (closed canopy) and savannah (open canopy). The main goals of this study were to (i) test the gain in using more than one acoustic recorder in a transect, (ii) measure the cost/efficiency relation of using one or more acoustic recorders in a transect, (iii) test the complementarity of two commonly used sampling methods, mist-nets and acoustic recorders. We tested for the first time in the Neotropics, the gain in species richness estimation of using more than one acoustic recorder, and its cost/efficient relation. Based on our results we present guidelines for further studies. The results showed a high complementarity of both methods in both open and closed habitats. This could also indicate that this double sampling strategy can maximize the efficiency of bat inventories, as we registered a high percentage of the region known bat species, with less sampling effort than other studies. Therefore, we think that this sampling strategy shows results that can contribute to the development of more efficient bat inventories worldwide. The standardizing of bat sampling in areas with similar bat communities can also increase the comparison of results between studies, facilitating the sharing of knowledge within the scientific community, and leading to better conservation plans on bats.

Keywords: Conservation; mist-nets; acoustic monitoring; Chiroptera; Amazonia; inventories

Resumo
O estudo de morcegos em todo o mundo pode ser feito recorrendo a uma grande variedade de métodos amostrais. Cada um desses métodos pode ser mais seletivo para certos grupos taxonómicos, ou grupos que se comportem ou se alimentem de forma ou comportamento semelhante (i.e. insectívoros, de voo alto ou baixo; frugíferos; cavernícolas; pernoitem em árvores, etc.). Esta variedade de métodos amostrais permite uma adaptação do esforço de amostragem resultando numa maior eficiência dos mesmos. No entanto, resulta também numa maior dificuldade de comparação com outros estudos, sendo eles realizados no mesmo sítio ou em sítios diferentes, devido a diferenças no método amostral. Isto dificulta a tomada de decisões informadas no que diz respeito à conservação de morcegos, particularmente relevante pois estes animais são não só importantes bioindicadores, mas também espécies chave nos serviços de ecossistemas, como por exemplo, na polinização e controlo de pragas.

Com o alto número de espécies consideradas ameaçadas ou não avaliadas pela UICN, uma das prioridades no estudo de quirópteros é o desenvolvimento de métodos amostrais estandardizados para realizar levantamentos de quirópteros, com o objetivo de facilitar a troca e comparação de informação sobre este grupo. O nosso estudo propôs um novo método amostral misto testado no escudo da Guianas (América do Sul), uma área com uma grande biodiversidade de quirópteros, permitindo assim a possibilidade de, (i) testar o ganho em utilizar mais do que um gravador acústico numa amostragem, (ii) testar pela primeira vez a relação custo/eficiência de utilizar mais do que um gravador acústico e (iii) testar a complementaridade entre os dois métodos utilizados (redes de neblina e gravadores de acústica).

O local escolhido para a realização deste estudo foram zonas de Cerrado do Amapá (Brasil), caracterizado por ser um habitat heterogéneo, constituído por zonas humanizadas (silvicultura, plantações de soja, etc.) e matrizes naturais de habitats que são caracterizados pela sua vegetação dominante (manchas de floresta de galeria, savanas, pradarias, etc.). Este local pertence à ecorregião do escudo das Guianas, consequentemente caracterizada por um alto nível de biodiversidade e endemismos. O clima qualifica-se como tropical de monção.

O desenho experimental utilizado para neste estudo consistiu na utilização de um método amostral misto (redes de neblina e gravadores de acústica) aplicado em 10 locais durante 4 noites cada (1 noite por mês entre junho e setembro). Cada local amostrado incluía uma área de Savana e uma mancha de Floresta (habitat de copa aberta e fechada, respetivamente). Em ambos os habitats as redes e os gravadores eram abertos/ligados em simultâneo, desde as ≈18:00h (pôr do sol) e durante 6 horas seguidas. Em cada habitat eram montadas em linha 9 redes de neblina, de 12×3 m e 14 mm de abertura de malha. A cerca de 50 metros, formando uma linha paralela à linha de redes, eram fixados, a 1,5 metros de altura e virados para cima, 3 gravadores separados aproximadamente 36 metros uns dos outros.

Devido à falha de algumas baterias nos gravadores de acústica perdemos algumas gravações. Tendo isto em conta decidimos optar por tratar os dados utilizando subamostras, retiradas dos dados que foram recolhidos com qualidade. Para a análise estatística construímos curvas de rarefação de espécies utilizando *Hill numbers*. Estimámos os *Hill numbers* correspondentes à riqueza de espécies, diversidades de *Shannon* e *Simpson*. Posteriormente, procedemos à comparação das curvas recorrendo à sobreposição dos intervalos de confiança estimados.

No que diz respeito ao ganho de ter mais do que um gravador por ponto de amostragem, todos os indicadores mostraram um aumento com o aumento do número de gravadores utilizados. No entanto, essa diferença só foi quase estatisticamente significativa (valor-p 0.06) para os valores de riqueza de espécies dos resultados conjuntos de manchas de floresta e savana. Em relação, aos custos de utilizar um, dois ou três gravadores, como seria de esperar os custos aumentam com o aumento dos gravadores, e a melhor relação custo/eficácia é a utilização de apenas um gravador, onde são gastos aproximadamente 92.40 € por espécie registada. Os dois métodos utilizados (redes de neblina e gravadores de acústica) tiveram resultados claramente complementares, sendo que todas as estimativas apresentam valores para ambos os habitats que atribuem aproximadamente 50% das espécies amostradas por gravadores e 50% por redes, com ligeiras variações dependendo do habitat. Apenas três espécies foram registadas por ambos os métodos na soma dos habitats, o que realça a sua complementaridade.

Apesar dos resultados descritos para a pergunta das vantagens em ter mais do que um gravador em cada ponto de amostragem, considerámos que seria necessário analisar os valores absolutos dos estimadores de riqueza. Percebendo que, não sendo estatisticamente diferentes na sua maioria, a utilização de mais do que um gravador por ponto poderia levar a um aumento das espécies estimadas. Sendo este facto mais relevante na situação contraria: a utilização de apenas um gravador poderia levar a uma menor riqueza estimada. Tirando partido do facto de este estudo também ter em consideração a relação custo benefício do método amostral, tivemos em consideração também estes valores para as análises e recomendações de estudos futuros. Para habitats de copa fechada, concluímos que o uso de três gravadores resulta num ganho de espécies estimadas comparado com o uso de um ou dois, mesmo que com gastos adicionais, o que poderá ser relevante quando avaliando a relevância para a conservação de um determinado local. Este resultado pode dever-se ao facto de espécies de morcegos insectívoros tenderem a voar acima da linha de copa em habitats fechados, fazendo com que apenas com um esforço maior (três gravadores) estas sejam registadas. Nos habitats de copado aberto concluímos que a utilização de dois gravadores apresenta resultados bastante semelhantes nas estimativas de riqueza de espécies quando comparado com a utilização de três gravadores, no entanto, apenas dois gravadores tornam-se a opção mais económica, das que não resulta numa perda de espécies estimadas. Isto é explicado pelo facto de os morcegos insectívoros tenderem a voar mais baixo em habitats abertos, o que faz com que, aliado ao facto de não existirem tantos obstáculos ao voo ou ao som, estes serem mais facilmente registados. Para a junção dos dados de ambos os habitats, estes comportam-se de forma semelhante aos do habitat aberto, no entanto, consideramos que este estudo deva ser replicado em habitats mistos para confirmar esta tendência.

No que diz respeito à pergunta da complementaridade dos dois métodos, confirmou-se o resultado esperado descrito em estudos anteriores de que estes dois métodos são altamente complementares e que mesmo em áreas bastante estudadas por um dos métodos, a utilização do outro resulta num aumento das espécies registadas para o local. No caso do nosso estudo podemos comprovar que, apenas em 22 transectos da nossa subamostragem para esta pergunta e restrito à área relativamente pequena abrangida pelo estudo quando comparada com o estado, registamos o equivalente a 73% das espécies alguma vez registadas em todo o estado do Amapá, o que serve como um indicador da excelente eficácia deste método para levantamento de uma comunidade de morcegos.

Para concluir, esperamos que este desenho amostral possa ser utilizado em outros locais, com habitats fechados (Floresta de *Terra Firme* ou Floresta Atlântica) ou abertos (Savana, Pradaria ou Pampa). Tendo este provado ser uma forma eficaz e económica para o levantamento de quirópterofauna e passível de aplicar à maioria de comunidades de morcegos do mundo. Levando a uma maior possibilidade de troca e comparação de informação sobre este grupo, melhorando assim a capacidade de resposta na conservação destas espécies.

Palavras-chave: Conservação; redes de neblina; monitorização acústica; Chiroptera; Amazónia; inventários

Index

List of Tables

List of Figures

1. Introduction

For centuries, bats have been misunderstood creatures that were victims of false popular beliefs. Their nocturnal habits and the existence of hematophagous species led many cultures to disdain and sometimes actively persecute this order (Chiroptera) of mammals for the most varied reasons (Knight 2008). However, with the progress in areas such as ecology, conservation and environmental education, people outside the scientific community are now more aware of the importance of bats to ecosystems (Bowen-Jones & Entwistle 2002; Ghanem & Voigt 2012). For example, the endemic Pemba's flying fox (*Pteropus voeltzkowi*) has been used as a flagship species for the protection of Pemba's island forest in Tanzania (Bowen-Jones & Entwistle 2002). Bats are bioindicators for a variety of environmental problems (Jones et al. 2009) and provide us with a wide range of ecosystem services (Kunz et al. 2011; Ghanem & Voigt 2012). However, many species are threatened and require conservation efforts that depend on solid scientific knowledge (Jones et al. 2009).

There are over 1400 bat species (Burgin et al. 2018), and over one third are classified as threatened (vulnerable, endangered or critically endangered) or *data deficient* by the International Union for Conservation of Nature (Frick et al. 2019). However, from those which are understudied (*data deficient*), nearly half belong to the Vespertilionidae family (IUCN 2020). This family is distributed in every continent except Antarctica, and most are high-flying insectivores (Corbet & Hill 1991; Neuweiler 2000; Zachos et al. 2020). They tend to fly mostly above the canopy, being more easily sampled with acoustic sampling (Meyer et al. 2011). With the rise of the number of threatened species and the existence of places that still do not have an acceptable inventory of bat species (Delgado-Jaramillo et al. 2020) there is a need of to design new sampling methods that are more time and cost effective (Meyer 2014).

There are habitats with potential to host high bat species richness that remain under sampled (Delgado-Jaramillo et al. 2020). This is particularly evident in the Brazilian part of the Guiana shield, where knowledge on the bat fauna is much scarcer than that available for other well-surveyed areas of the same region (Lim & Tavares 2012). The areas along the Amazon river have been better studied and inventoried than more remote areas (Bernard et al. 2011). It is noteworthy that most of the bat surveys use mist nets as the only sampling method, which could result in a sampling bias (Flaquer 2007; Silva & Bernard 2017). Carrying out this study in a rich environment as the Amazonian Guiana Shield, with such a diverse bat fauna (Santos 2019), can encourage others to use more complete sampling methods in other habitats.

FAO 's report (FAO 2010) pointed out that tropical forests in the 2000s decade showed a decreasing deforestation rate, when compared with the 1990s. However, this was contradicted by Kim (2015) who presented data that indicating an intensification in forest loss. Nevertheless, both of them referred that high rates of tropical deforestation were happening; Kim (2015) just showed a much more alarming trend, as this rate was accelerating in the tropical region. Tropical rainforests with mature forest patches are essential for the survival of many rare species (Watson et al. 2018). Alerting the scientific community and the general public for the need to reverse this trend is an ever more urgent requirement. It is important to note that the high rate of tropical deforestation can result in extinction events that go unnoticed, as a consequence of the incapability to inventory and monitor tropical disturbed forests (Alroy 2017).

Deforestation of tropical regions can be done legally, i.e., for legal crops or livestock farming, or illegally (i.e., illegal logging). However, fact that deforestation is legal does not mean that it is sustainable. As highlighted by Carvalho (2019) in the Brazilian native forest of the Amazonian and Cerrado biomes, the soy planters or cattle ranchers often find ways to evade government regulations. In order to invert this tendency, governments, private sector and society must act. This action is only viable when and where there is knowledge to make the most adequate decisions, so the scientific community should make efforts to facilitate knowledge transfer to society and decision makers. Empowered with better knowledge they can make informed decisions, more likely to result in successful conservation efforts (Barlow et al. 2018).

Many authors advocate the need to standardize the sampling of bats (Jones et al. 2009; Meyer et al. 2011; Stahlschmidt & Brhül 2012; Ghanem & Voigt 2012; Arias-Aguilar et al. 2018). The need of finding sampling methods that are cost and time efficient is increasingly more pressing, to facilitate the development of more comparable studies between regions.

The use of acoustic sampling in tropical bat communities, particularly in neotropics, remains infrequent (Meyer 2014). However, there is already some work in the field of acoustic sampling standardization (Stahlschmidt & Brhül 2012). Until fairly recently the costs of acoustic sampling were very high, as just the purchase of one recording unit could reach 3,000 €. However, the recent release of a new low-cost acoustic sampler may greatly facilitate the use of acoustic techniques in large scale fauna assessments (Hill et al. 2018).

In the Neotropics, phyllostomid bats (family Phyllostomidae) are captured more often in mist nets than high-flying insectivorous bats (e.g., Molossidae and Vespertilionidae families). The latter, are better sampled using acoustic recorders or by using mist nets at the exit of shelters or roosts, (MacSwiney et al. 2008). Most bat surveys in the Neotropics only use mist net sampling, so their results tend to be strongly biased to phyllostomid bats (Delgado-Jaramillo et al. 2020). Due to flying over the canopies, high-flying insectivorous bats (MacSwiney et al. 2008; Marques et al. 2016) usually avoid mist-nets, thus appear rarer than they really are (Meyer et al. 2011). This could result in a misinterpretation of the real importance of some species on the ecosystems.

The use of acoustic recorders in addition to mist-nets, resulted in some studies in the identification of 30% more species than simply using mist-nets, particularly aerial insectivores, in both the Neotropics (MacSwiney et al. 2008) and southeast Asia (Furey et al. 2009). This confirms that even relatively common species can be easily overlooked when sampling with only one method (Flaquer 2007; Meyer et al. 2011).

Realizing the importance of bats for the ecosystems and the need to develop new efficient and inexpensive ways to inventory bats, we decided to test a new sampling strategy. We assess, for the first time in a tropical region, the complementary of a low-cost acoustic recorder (Audiomoth®) and mist nets for sampling bats. Additionally, this is the first study in the Amazonian forest to investigate the cost-efficiency of using different numbers of acoustic recorders for surveying bats in open and closed habitats. We expect that our findings are possible to extrapolate to other closed and open habitats, i.e. *Terra firme* forests and Atlantic forest or *Cerrado* and pampas. Eventually, the results could also be extrapolated to tropical biomes in other continents (MacSwiney et al. 2008; Furey et al. 2009).

The aim of this thesis was to contribute to the development of methods to sample bats, using mist nets and acoustic recorders in a savannah matrix and in forest patches in *Cerrado do Amapá,* in the north of the Brazilian Amazon. Specifically, the objectives were: (i) test the gain of using one, two or three acoustic recorders at the same time in savannah and in forest; (ii) estimate the cost-efficiency ratio of registering each species according to the number of acoustic recorders used in savanna and in forest; and (iii) verify the complementarity of sampling using mist nets and acoustic recorders in the savannah and in the forest. We hypothesized that (i) as in other sampling methods (Bergallo et al. 2003), more species would be recorded with higher sampling effort; (ii) the cost-efficiency of the acoustic sampling, as assessed by the gain in new species, will increase the efficiency with the use of more acoustic recorders. However, since we expect a raise in species registered with more acoustic recorders, the cost per species is expected to lower or maintain; and (iii) there is a high complementarity between the two sampling methods (MacSwiney et al. 2008; Furey et al. 2009), with mist nets detecting a greater number of phillostomids (Marques et al. 2016; Delgado-Jaramillo et al. 2020) and the acoustic recorders registering a greater number of insectivorous bats (Meyer et al. 2011).

2. Materials and methods

2.1. Study area

This study was carried in the Cerrado do Amapá, an Amazonian savannah located on the eastern edge of the state of Amapá, in the north of the Brazilian Amazon (Figure 2.1). The Cerrado do Amapá can be subdivided into areas dominated by one of four main vegetation types: shrub savannah, woodland savannah, grass savannah, and park savannah (Mustin et al. 2017). Along the Cerrado do Amapá, these different types of savannah form a matrix that contains natural forest patches, gallery forests, seasonally flooded areas, and *buritizais* (monodominant gallery forest of *Mauritia flexuosa*) (Mustin et al. 2017). Due to its complexity and heterogeneity, the Cerrado do Amapá is home to over 370 species of plants, 350 species of invertebrates and 400 of vertebrates (Mustin et al. 2017). At least 60 species of bats have been detected in the savannahs and forest patches of the Cerrado do Amapá (Silva et al. 2013; Mustin et al. 2017; Carvalho et al. 2018; Luz et al. 2019). This region has a high richness of bat species compared to the rest of Brazil (Delgado-Jaramillo et al. 2020) and is considered a high priority for sampling of mammals (Martins et al. 2011; Silva et al. 2013), and in particular bats, one of the least sampled mammalian taxa in the region (Martins et al. 2011). In fact, the main gap in the study of bats in this region relates to the lack of acoustic surveys targeting insectivorous bats.

The *Cerrado do Amapá* has a total area of ~140,012 km^2 (Mustin et al. 2017), and ~1,949 km^2 (~19.5% of Cerrado do Amapá) have been altered for use in silviculture, mechanized agriculture, livestock production and exploration of mineral resources (GEA 2016). Currently, the *Cerrado do Amapá* is the most threatened complexes of Amazonian savannah in Brazil (Carvalho & Mustin 2017). The main threat that occurs in these South American savannahs is linked with vegetation clearing for the cultivation of grains and legumes (mainly soybeans and maize), and plantations of eucalyptus and acacia trees (Carvalho & Mustin 2017; Carvalho et al. 2019). Specifically, in our study area, near the capital of the state of Amapá, Macapá, the *Cerrado do Amapá* is still little altered, but large areas have been replaced by soy plantations in the last five years.

This region has a Tropical monsoon climate (according to Köppen's climate classification), with rainfall of the driest month below 60 mm and annual rainfall ranging from 2,300 to 2,800 mm (Souza & Cunha 2010). The rainy season runs from December to July, the dry season runs from August to November, and the average air temperature does not have a large thermal range, oscillating around 27°C. (Tavares 2014).

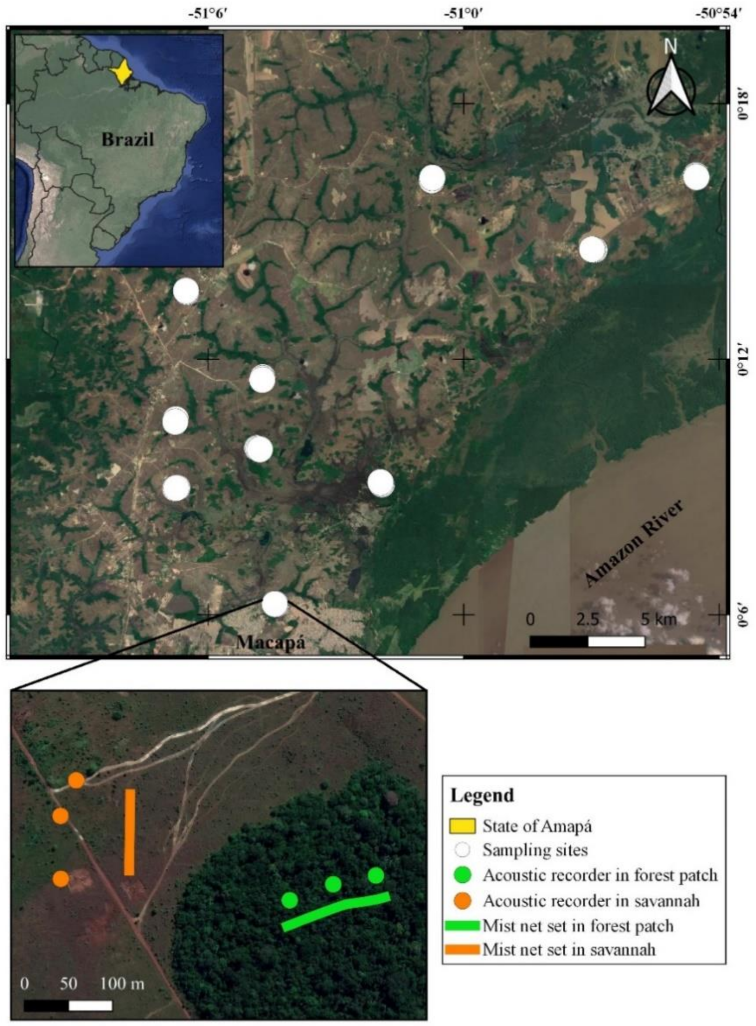

Figure 2.1 – Location of sampling sites (Amapá, Brazil)(top), and example of sampling at these site, with mist-nets lines and acoustic recorders in both open and closed habitats.

2.2. Sampling design

In the central region of Cerrado do Amapá, close to the state capital, Macapá, we selected 10 sample sites. Each of these sites had one transect in natural forest patches and one transect in savannah areas (Figure 2.1), totalling 20 transects. All locations chosen were natural, with little human intervention (e.g., vegetation clearing). The study area was located in an area dominated by park savannah (Mustin et al., 2017), which is characterised by an open tree storey that does not pass three metres in height, having a very open canopy (Costa-Neto 2014). The forest patches in our study region, which are embedded in the savanna matrix (Mustin et al. 2017), have a closed canopy, and trees with an average height of six meters, with scattered trees reaching up to 25 m.

2.3 Bat sampling

We conducted four surveys in each site in four consecutive months, two in the wet (June – July 2018) and two in the dry season (August – September 2018). Each survey included simultaneous sampling in the transect located in both forest patches and savannah, at each site. This resulted in a total of 40 days of sampling in forest patches and 40 days in savannah.

In each transect, we used nine mist-nets (12×3 m; 14 mm mesh size) to sample bats, set in the understory along a ~110 m trail (e.g., Luz et al. 2019). The mist-nets were fixed in sequence and at least 30 meters from the edge between the two different habitats (forest and savannah), in order to minimize the influence of edge effects on the capture of bats (e.g., Meyer et al. 2016). In addition, three acoustic recorders (AudioMoth® - Hill et al. 2018) were placed at approximately 1.5 m height, forming a line parallel to the nets and at least 50 m from it. We used a distance of at least 50 meters from the mist-nets, because if the recorders were closer to the mist-nets, the trapped bats could be recorded. For the recorders to cover a distance of approximately the same as the mist-nets, each one was fixed at a distance of approximately 36 meters from each other. Both methods were left-open/recording for 6 hours since sunset (~18:00h to 00:00) and the mist-nets were revisited every 20 minutes. The recorders were configured to record a frequency of 384 kHz, medium gain and to record continuously and save the sound file every five minutes. The mist-net sampling effort, calculated accordingly to Straube and Bianconi (2002) resulted in a total 155.520 m^2.h (77.760 m^2.h in forest patches and 77.760 m^2.h in savannahs).

Every bat captured in the mist-nets was placed in a cotton bag and then measured and identified. We identified the species, age and sex, measured the forearm and body mass, and marked each animal with a necklace with numbered metal or plastic rings in adults (see Esbérard & Daemon 1999) and a small puncture in the propatagium in juveniles (see Bonaccorso & Smythe 1972) in order to quantify recaptures. All the recaptures during the same night were excluded for this work. The bats were identified in the field according to Lim and Engstrom (2001), Gardner (2008 [2007]), Reis et al. (2017) and Lòpez-Baucells et al. (2018). Nomenclature of species follows Nogueira et al. (2018). Fieldwork, handling and processing of all captures at all study sites followed the guidelines of the American Society of Mammalogists (Sikes et al. 2011).

Recorded calls were identified using the software Kaleidoscope. With this software we run an automatic process to split the records of all nights sampled, that were previously stored in five minutes Waveform Audio File Format (.WAV). Each of those, was divided into multiple 5 seconds WAV files (in order to ease the following labelling), files without sound or with only noise were discarted. Files were discarded if they did not include at least two pulses with the following characteristics: between 10 and 200 kHz as minimum and maximum frequency, with a minimum time length of 2 ms and a maximum of 500 ms, and a maximum inter-syllable gap of 500 ms. For the manual identification of the bat calls we used all the pulse characteristics available, such as the shape of the pulse, its duration, bandwidth, and frequency of maximum energy. In some species it was necessary to see which was the harmonic with the frequency of maximum energy, and to observe the pulses before and after the one to be analysed, because some species produce a specific sequence of different pulses. The identification followed the instructions described in Lòpez-Baucells et al. (2018) and Arias-Aguilar et al. (2018) and was done to the lowest taxonomic level possible. Whenever possible, bat calls were assigned to a species, otherwise they were assigned to sonotypes, i.e. groups of species that emit calls that are very difficult or even impossible to distinguish with acceptable certainty.

2.4 Data analysis

Due to some problems with the batteries of the acoustic recorders we lost a total of 71 out of 240 acoustic registers (i.e. 6 hours of one acoustic recorder in a night). In 27 out of the 40 nights, at least one acoustic recorder did not record. We decided to make a subsample, for each of the questions, using only the fully functional acoustic recorders, and only with the nights of the rainy season (which had a higher probability of recording a higher number of species for our study area (Carvalho et al. 2018). All the mentioned analyses were made in the program R (R Core Team 2020).

To investigate whether there is a gain in using more than one acoustic recorder per transect, we used a total of 15 transect visits, eight in forest patches and seven in savannah. From the data of incidence (presence and absence) for one, two or three acoustic recorders, considering forest patches and savannah together and separately, we constructed rarefaction species accumulation curves in the 'iNEXT' R package (Hsieh et al. 2016) using Hill numbers. We estimated the Hill numbers corresponding to the species richness (q = 0), the exponential of Shannon's entropy index (q = 1 – later called Shannon diversity), the inverse of Simpson's concentration index (q = 2 – later called Simpson diversity) for one, two or three acoustic recorders by transect. Hill numbers q = 0 is simply species richness, q = 1 can be interpreted as the effective number of common bat species in the assemblage (or common bats species sampled by a method), and q = 2 as the effective number of dominant species in the assemblage (or dominant bat species sampled by a method). The rarefaction curves were made up to the limit of sampling units for each of the analysed habitats (eight in forest patches and seven in savannah). Subsequently, comparisons of the curves between habitats were made based on the overlap of the estimated confidence interval (see Chao and Chiu 2016). To verify the overlap between the confidence intervals of the rarefaction curves, we made a function in the program R. In addition, we compared rarefaction curves (Cayuela et al. 2015) through the 'rareNMtests' R package, using null models (Cayuela & Gotelli 2015).

Furthermore, and considering the monetary costs spent in this work and the species identified, we calculated the cost to identify each species using three, two or one acoustic recorder per transect. The cost was estimated in Brazilian Real (R$) and then converted to Euros (€), with a conversion rate of 1 R$ to ≈ 0.21€, the conversion rate at 30 of September of 2018, when the field sampling ended.

In the estimation of expenses, we considered both fixed and variable expenses. The fixed expenses (researcher and field assistant salary, external hard disk, fuel, etc.) were considered all those that did not varied depending on the number of acoustic recorders used per transect. The variable expenses (alkaline batteries, secure digital cards, salary of the researcher that identified the acoustic data, etc.) were considered all those who varied depending on the number of acoustic recorders used.

To investigate the complementarity of mist-nets and acoustic recorders, we made a subsample with 22 visits to transects, twelve in forest patches and ten in savannah. Here, we also constructed rarefaction curves for species based on Hill numbers (q = 0, q = 1, and q = 2) and made comparisons between the curves as described above. In addition, we verified the proportion of species that each of the methods registered in relation to the total richness. For all analyses, we also considered forest patches and savannah both together and separately.

Table 2.1 – Species captured with mist-nets (left) and species/sonotypes identified with acoustic sampling (right), divided by forest patches (FP) and Savannah (SV).

Family and species/sonotypes	Mist-nets		Acoustic recorders	
	FP	SV	FP	SV
Emballonuridae				
Centronycteris centralis/maximiliani	-	-	X	X
Cormura brevirostris	-	-	X	X
Cyttarops alecto	-	-	-	X
Diclidurus sp.	-	-	-	X
Diclidurus albus/scutatus	-	-	X	X

Diclidurus ingens	-	-	-	X
Peropteryx sp.	-	-	-	X
Peropteryx kappleri	-	-	X	X
Peropteryx leucoptera	X	-	-	-
Peropteryx macrotis	-	-	X	X
Peropteryx pallidoptera	X	-	-	-
Peropteryx trinitatis	-	-	X	X
Rhynchonycteris naso	X	-	-	-
Saccopteryx sp.	-	-	X	X
Saccopteryx bilineata	-	X	X	X
Saccopteryx canescens/gimnura	-	-	X	X
Saccopteryx leptura	X	-	X	X
Molossidae				
Molossidae sonotype 1	-	-	X	X
Molossops sp.	-	-	X	-
Molossops neglectus	-	-	-	X
Molossus sp. sonotype 1	-	-	X	X
Molossus sp. sonotype 2	-	-	X	X
Molossus Molossus	-	X	X	X
Promops sp.	-	-	X	X
Promops centralis	-	-	X	X
Promops nasutus	-	-	-	X
Phyllostomidae				
Ametrida centurio	-	X	-	-
Artibeus concolor	X	X	-	-
Artibeus lituratus	X	X	-	-
Artibeus obscurus	X	X	-	-
Artibeus planirostris	X	X	-	-
Carollia brevicauda	X	X	-	-
Carollia perspicillata	X	X	-	-
Chiroderma trinitatum	X	-	-	-
Dermanura cinerea	X	X	-	-
Dermanura gnoma	-	X	-	-
Desmodus rotundus	X	X	-	-
Glossophaga soricina	X	-	-	-
Lophostoma brasiliense	-	X	-	-
Lophostoma silvicola	X	-	-	-
Macrophyllum macrophyllum	-	X	-	-
Micronycteris microtis	X	-	-	-
Micronycteris minuta	-	X	-	-
Micronycteris schmidtorum	X	-	-	-
Mimon crenulatum	X	X	-	-
Phyllostomus elongatus	X	-	-	-
Phyllostomus hastatus	X	-	-	-
Platyrrhinus sp.	-	X	-	-

Platyrrhinus fusciventris	-	X	-	-
Platyrrhinus incarum	X	X	-	-
Rhinophylla pumilio	X	X	-	-
Sturnira lilium	X	X	-	-
Tonatia saurophila	X	-	-	-
Trinycteris nicefori	-	X	-	-
Uroderma bilobatum	X	X	-	-
Uroderma magnirostrum	-	X	-	-
Vampyriscus brocki	-	X	-	-
Vespertilionidae				
Vespertilionidae sonotype 1	-	-	-	X
Eptesicus sp.	-	-	X	-
Lasiurus sp.	-	-	X	X
Myotis nigricans	-	-	X	X
Myotis riparius	X	-	-	-
Rhogeessa sp.	-	-	X	X
Total	26	25	21	26
Total method	38		28	

3. Results

Overview for acoustic recorders and for mist nets

From the data of 22 transects, 12 in forest and 10 in savannah, sampled with acoustic recorders, we identified 23,363 bat passes of 28 call types and 625 (≈ 2,7 %) bat passes with not enough quality to be classified. The latter will not be considered for ensuing analysis. Of that total, 11,015 bat passes were identified in forest patches, belonging to 24 different call types (Table 2.1). The other 12,348 bat passes from the savannah resulted in 26 call types (Table 2.1).

In the 80 transects of mist nets we captured a total of 973 bats of 54 species. For the data used to answer the question of complementarity between mist nets and acoustic recorders we used a total of 322 bats captured in 12 transects in forest and 10 in savannah, with 228 bats captured in forest patches and 97 in savannah (Table 2.1). Savannah showed an observed species richness slightly higher than the forest patches (33 vs 30 species), with 17 exclusive species, five of which were insectivores (Table 2.1). Forest had 14 exclusive species, five of which were insectivorous.

3.1. Gain from having more than one acoustic recorder in a single transect

Based on data of 15 transects, eight in forest patches and seven in savannah, we identified a total of 17,612 bat passes and 28 sonotypes. Of that total, 9,131 bat passes were identified in the forest patches belonging to 20 different sonotypes. The other 8,481 bat passes from savannah were assigned to 27 sonotypes. Only one species – a *Molossops* sp. (not possible to identify to species level) – was exclusive of the forest patches. However, in the savannah we identified 3 bat passes of *Molossops negletus*.

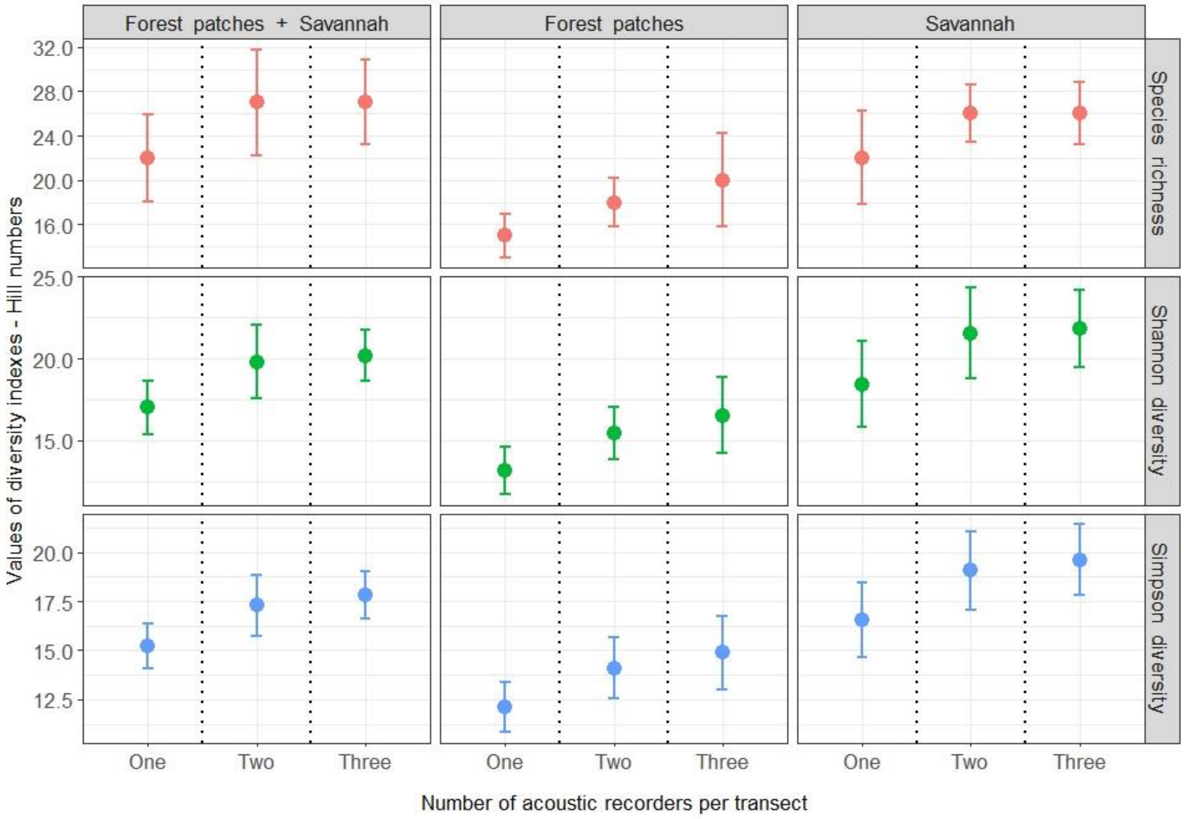

Figure 3.1– Diversity values for species richness (Hill numbers - q = 0), Shannon diversity (Hill numbers - q = 1) and Simpson diversity (Hill numbers - q = 2) of bats sampled in transects with one, two or three acoustic recorders in forest patches and savannah matrix in Cerrado do Amapá, north of the Brazilian Amazon. Vertical lines represent 95% confidence intervals.

Pooling forest patches and savannah together, the estimated species richness was 22 species, using one acoustic recorder per transect, and 27 species using two or three acoustic recorders per transect (Figure 3.1). For Shannon and Simpson diversity, the values were higher considering three acoustic recorders (20.18 and 15.21, respectively – Figure 3.1), followed by two acoustic recorders (19.8 and 17.29, respectively) and one acoustic recorder (17 and 17.80, respectively). However, there was no difference between the use of one, two or three acoustic recorders for species richness, Shannon and Simpson diversity, when comparing the curves (Table 3.1), and all confidence intervals were overlapping (Figure 3.2). For forest patches, the use of three acoustic recorders led to higher species richness, Shannon and Simpson diversity values (20; 16.53; and 14.88, respectively – Figure 3.1) than the use of two (18; 15.45; and 14.10, respectively – Figure 3.1) or one acoustic recorder (15; 13.14; 12.12 – Figure 3.1). Savanah followed this same pattern, with species richness, Shannon and Simpson diversity being higher when using three acoustic recorders (26; 21.83; and 19.61, respectively – Figure 3.1), followed by two acoustic recorders (26; 21.56; and 19.07, respectively – Figure 3.1) and one acoustic recorder (22; 18.42; and 16.53, respectively – Figure 3.1). However, for both forest patches and savannah, all confidence intervals overlapped (Figure 3.2) and there was no difference between the diversity curves when using one, two or three acoustic recorders (Table 3.1). Note that the values of species richness for three and two acoustic recorders had the same values (26; Figure 3.1) and the similarity between the corresponding values of Shannon and Simpson diversity was higher than observed in forest patches.

Table 3.1 - *P* values for the ecological null models test comparing the species richness (Hill numbers - q = 0), Shannon diversity (Hill numbers - q = 1) and Simpson diversity (Hill numbers - q = 2) of bats sampled by one, two or three acoustic recorders per transect located in forest patches and savannah matrix in Cerrado do Amapá, north of the Brazilian Amazon.

	Number of acoustic recorders	Species richness	Shannon diversity	Simpson diversity
Forest patches + Savannah	1 x 2	0.13	0.33	0.26
	1 x 3	0.06	0.26	0.13
	2 x 3	0.53	0.60	0.80
Forest patches	1 x 2	0.50	0.50	0.62
	1 x 3	0.12	0.25	0.12
	2 x 3	0.87	0.75	0.75
Savannah	1 x 2	0.14	0.14	0.42
	1 x 3	0.14	0.28	0.28
	2 x 3	0.28	0.85	0.85

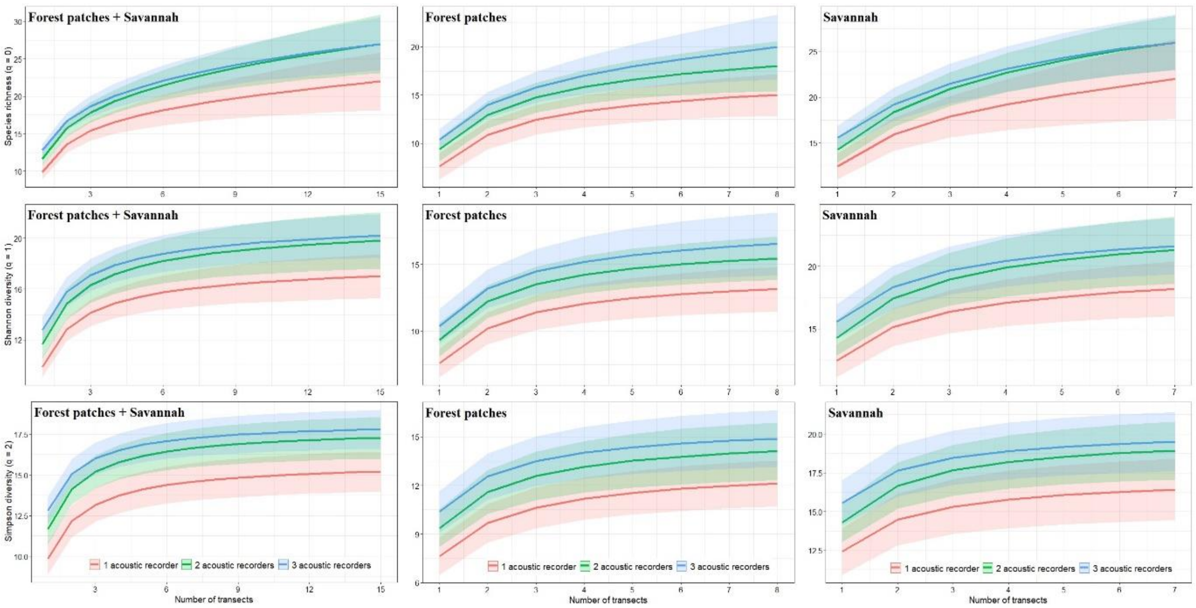

Figure 3.2 - Rarefaction curves for species richness (Hill numbers - q = 0), Shannon diversity (Hill numbers - q = 1) and Simpson diversity (Hill numbers - q = 2) of bats sampled by one, two or three acoustic recorders per transect in forest patches and savannah matrix in Cerrado do Amapá, north of the Brazilian Amazon. The shaded area represents the 95% confidence interval.

3.1.1 Monetary cost of using multiple acoustic recordings

We found that total spending on identification, when using three acoustic recorders for both habitats, was ≈5,885.04 € (≈R$ 28,024.00), and when divided by the number of species identified, the total cost was of ≈210.00 € (≈R$ 1,000.00) per species. The total cost estimated, when using two acoustic recorders was ≈4,518.36 € (R$ 21,516.00), that is, a reduction of ≈23%, and the cost per species was ≈102.69 € (R$ 489.00), with a reduction of ≈51% comparing with the use of three acoustic recorders. When we consider only one acoustic recorder, the total cost was ≈3,361.68 € (R$ 16,008.00), reducing the cost by ≈43% and for ≈56% per species with ≈92.40 € (R$ 440.00) comparing with three acoustic recorders. When we compare the costs between having one or two acoustic recorders, we found that with a single recorder we had a total reduction of ≈26% (≈ 1,156.68 € / R$ 5,508.00) in total costs and ≈10% (≈10,29 € / R$ 49.00) in cost per species.

Table 3.2 – Per species costs by habitat, considering one to three acoustic recorders.

	Per species cost - € (R$)	
	forest patches	savannah
1 acoustic recorder	100 (478)	84 (402)
2 acoustic recorders	103 (490)	102 (488)
3 acoustic recorders	115 (549)	138 (655)

3.2. Complementarity of mist nets and acoustic recorders in bat sampling

From the data of 22 transects analysed to study the complementarity between the methods, pooling forest patches and savannahs, we captured a total of 332 individuals belonging to 38 species using mist nets, and 28 unique sonotypes from 16,973 bat passes using the acoustic recorders.

Considering only forest patches, we recorded 26 species from 225 captures made with mist nets, and 21 sonotypes from 8,376 bat passes recorded by acoustic recorders. For the joint results of mist nets and acoustic recorders in forest patches we had 46 species/sonotypes (*Saccopteryx leptura* was registered in both methods).

For the savannah, we recorded 25 species from 97 captures made with mist nets, and 26 sonotypes from 8,597 bat passes recorded by acoustic recorders. Resulting in a joined result of 49 species/sonotypes (*Molossus molossus* and *Saccopteryx bilineata* were registered in both methods)

Analysing the rarefaction curves, we can observe that both methods represented approximately half of the diversity estimators' proportion, either in forest patches, savannah and joined results (Figures 4 and 5). In addition, proportionally, the mist nets sampled more species and higher diversity in forest patches than in savannah. In the latter, the acoustic recorders sampled more species than mist nets (Figure 3.4).

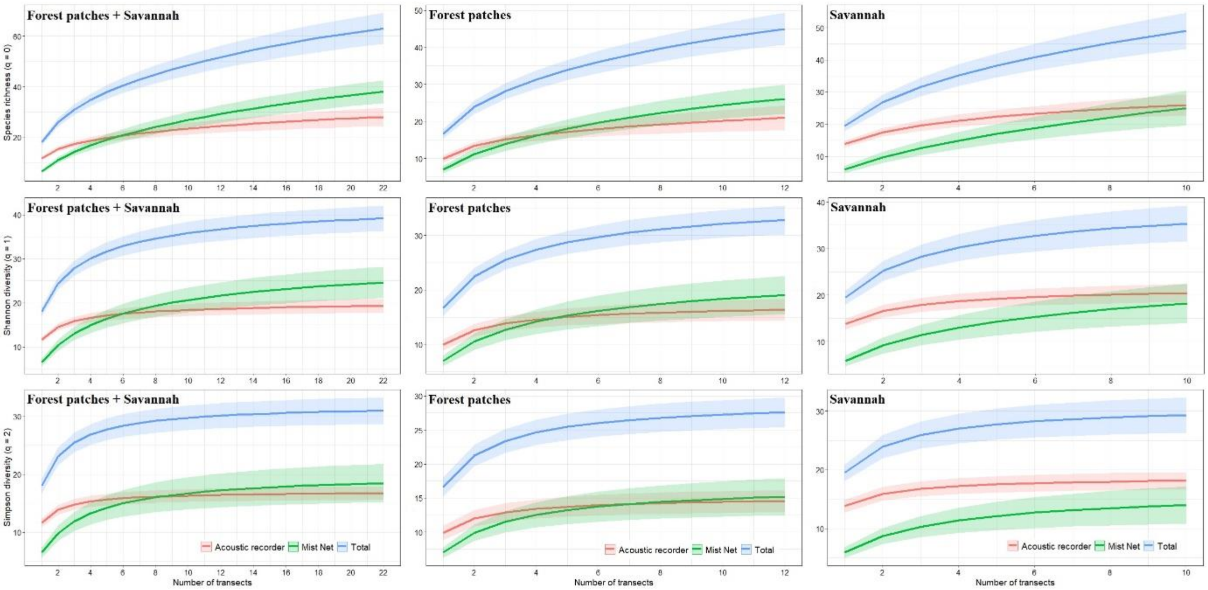

Figure 3.3 - Rarefaction curves for species richness (Hill numbers - q = 0), Shannon diversity (Hill numbers - q = 1) and Simpson diversity (Hill numbers - q = 2) of bats sampled by mist nets and acoustic recorders in forest patches and savannah matrix in Cerrado do Amapá, north of the Brazilian Amazon. The shaded area represents the 95% confidence interval.

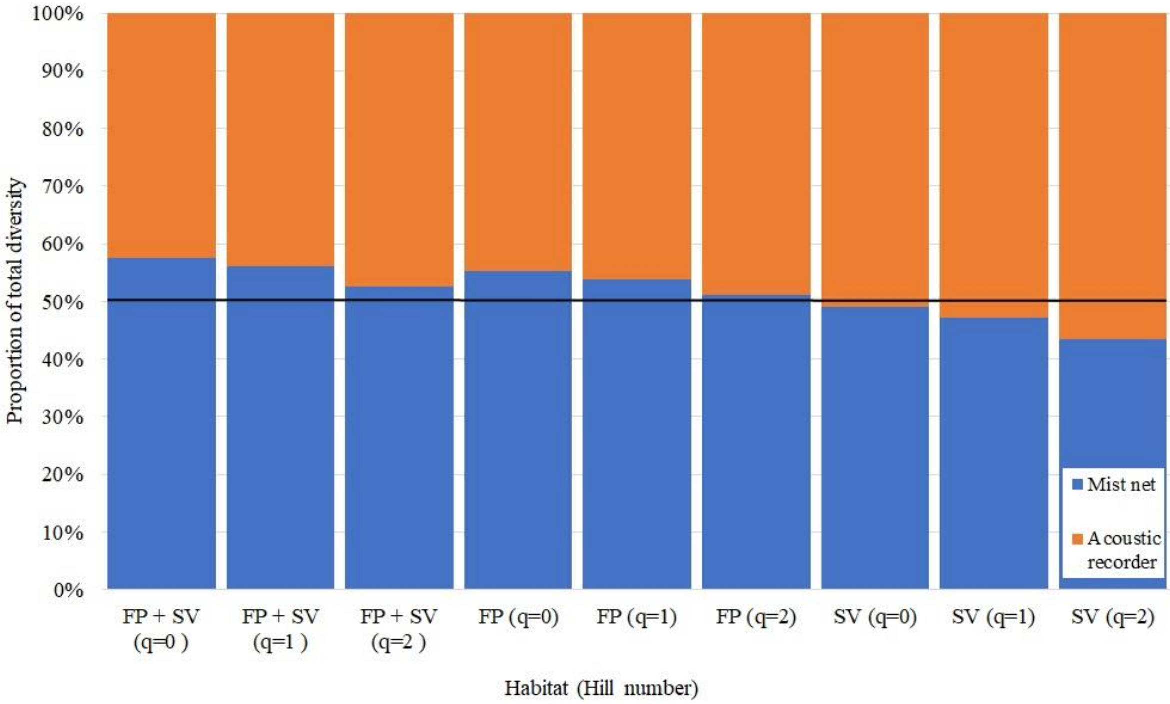

Figure 3.4 – Proportion of total diversity for species richness (Hill numbers - q = 0), Shannon diversity (Hill numbers - q = 1) and Simpson diversity (Hill numbers - q = 2) of bats sampled by mist nets and two acoustic recorders in forest patches and savannah matrix in Cerrado do Amapá, north of the Brazilian Amazon. The black horizontal line indicates the proportion of 50% of total diversity.

4. Discussion

Our evaluation of the gain of using more than one acoustic recorder per transect (3.1) did not show a statically significant difference (p-value < 0.05) between using one or more recorders in any of the diversity values (Table 3.1). However, we detected a relation between gain of species and sampling cost, which shows that inventories can maximize the species sampled without reaching unnecessary monetary costs. In the evaluation of the complementarity of mist nets and acoustic recorders (3.2), we registered 46 species/sonotypes exclusively in forest, and only one species was registered by both methods. Moreover, in the savannah we registered 49 unique recordings with two species being registered by both methods. With these results, we conclude that combining both methods greatly improve species inventories.

4.1. Gain from having more than one acoustic recorder per transect

The statistical analysis of the ecological null model test showed no statistically significant differences when comparing the diversity values (Table 3.1). However, pooling forest patches and savannah the values of species richness using three acoustic recorders were higher than using a single acoustic recorder (near significant, p-value = 0.06). Despite of the statistical results (Table 3.1), we can observe differences in the absolute diversity values. These differences may lead to underestimations or poor estimations of local diversity, when we would choose to use one or more acoustic recorders, which in some cases, could mislead the allocation of conservation efforts (Vaughan & Ormerod 2005). In addition, there are still biodiversity hotspots of vertebrates, previously thought to be well studied, with proven major underestimations in species richness (Vieites et al. 2009).

In closed habitat (forest) the use of one acoustic recorder resulted in the estimation of 15, 13.14 and 12.12 for species richness, Shannon and Simpson diversity respectively, while the use of three acoustic recorders resulted in 20, 16.53, and 14.88, respectively (Figure 3.1), which is summarized in a difference of 5, 3.39 and 2.76 in the species richness and diversity indexes for the same study area. As for the open habitat (savannah), between one and two acoustic recorders, the difference was of 4, 3.14 and 2.54 for species richness, Shannon and Simpson diversity respectively, and 0, 0.27 and 0.54 for the difference between two and three acoustic recorders. Due to the lower difference between the use of two and three acoustic recorders, we analysed the sampling cost per species surveyed in both cases. With two acoustic recorders the sampling cost per species was 102 € (488 $R), contrasting with the 138 € (655 $R) per species using three acoustic recorders. The results for the joined data of open and closed habitat (forest and savannah) were similar to the observed in closed habitat (Figure 3.1). As this was the first study to our knowledge to compare the gain or difference of adding more than one acoustic recorder to each sampling transect, and, based in our results, the use of three acoustic recorders in a closed habitat transect, result in the higher estimated species richness. This could be due to the height at which our acoustic recorders were placed (≈1.5 meters), which could have influenced the detection of insectivorous bats, that tend to forage at higher canopy heights when in closed habitats (Marques et al. 2016). This circumstance may result in a lower detectability of bat calls at the ground level in forest, explaining that some may only be detected when using three acoustic recorders. In this case, it is the flying behaviour that can influence the species detectability. The use of only two acoustic recorders in open habitats, as the option that presents itself as the best cost/efficiency ratio (that do not result in a decrease in richness estimation), should be explained by the absence of obstacles in that habitat, which result in a decrease of bat's flight height (Meyer et al. 2011). Also, tree cover can be used as a good predictor to prey density (Müller et al. 2012) and bats are known to compensate call intensity when more distant to prey (Surlykke & Kalko 2008), resulting in higher probability of producing detectable calls in habitats with less prey and where there are no obstacles that can attenuate echolocation pulses (Fricke 1984). As for the joined results of open and closed habitats, we advise caution in the interpretation of these results, since they could not be a good predictor of this sampling strategy performance in habitats with irregular canopy density. Instead, they could act as an indicator of the overall species richness in the sampled area when applying this sampling strategy in multiple habitats, as it happens in our study.

4.2. Complementarity of mist nets and acoustic recorders in bat sampling

Our study points out that, in closed habitat, the mist nets detected slightly over 50% of species and in the open habitat the acoustic recorders detected slightly over 50 % of the species (Figure 3.4) as well. We recorded only three species with both methods. These results indicate that these two sampling methods have a high complementarity, as observed before (Flaquer 2007; Silva & Bernard et al. 2017). In the subset of data used in this question we registered a total of 63 species/sonotypes of bats, with only 22 transects sampled. The effort per transect was 1994 m^2.h of nets and two acoustic recorders. Consequently, the efficiency of this sampling strategy, when it refers to species registered, becomes noteworthy when compared to other studies conducted in the same region, that used only mist nets (Martins et al. 2006; Martins et al. 2011; Castro & Michalsky 2015). Thus, revealing that the protection measures indicated in those same studies could be even more relevant to protect the local biodiversity than previously thought. Since they may only have registered close to 50% of the bat fauna that they could potentially have inventoried, as if they had used acoustic sampling, or may have considered insectivorous bat species (more easily registered with acoustic sampling) as rare species with low impact in the ecosystem, where they could possibly be common and extremely relevant. Additionally, in only 22 transects within a small area (Figure 2.1) we were able to sample approximately 73% of the 86 species described in the state of Amapá (Martins et al. 2006; Martins et al. 2011; Silva et al. 2013; Castro & Michalsky 2015)

Recalling that we collected acoustic and capture data, in each transect at the same time, this strategy becomes a viable alternative for bat sampling in cost and time spent. This is particularly important for long-term studies or when monitoring with limited funding (Caughlan & Oakley 2001). Moreover, the characteristics of a habitat, such as canopy cover, tree density and vertical stratification, highly impact the foraging behaviour of bats (Kusch et al. 2004; Müller et al. 2012; Rocha et al. 2016; Kerbiriou et al. 2019) and thus their detectability. Although we recommend that other studies should try to replicate our sampling strategy, we expect that our results are possible to extrapolate to other closed and open habitats, like *Terra Firme* or Atlantic forest habitats and grasslands or Pampas.

5. Conclusion

This study evaluates a new mixed sampling strategy for the study of bats and proved that there is a high complementarity between the methods used – mist-netting and acoustic recording. The strategy was tested in two types of habitats in one of the richest hotspots of bat diversity (Delgado-Jaramillo et al. 2020). The two methods that we used may not be always quite as complementary as they are in our study region, especially where Phyllostomids are absent or represent a small part of the bat assemblages. As in most of the neotropics, phyllostomids are an important part of the bat assemblage in our study area and because they are relatively easy to mist-net they are heavily represented in the capture results. However, their calls are very hard to record, so they are nearly absent from the results of the acoustics. As a consequence, the results of the two methods are very different and complementary. We recommend that the complementarity of using acoustic and capture methods should be tested in other biomes with different bat communities, in order to understand if applying both methods is an important strategy to sample their bat assemblages. We would want to highlight the importance that the possibility of comparing different studies has for the progress in scientific knowledge (Ghanem & Voigt 2012; Jones et al. 2009) as well as the importance of a well-managed budget has in designing surveys. The lack of knowledge on bat species assemblages in some regions that were already sampled in the past (Bernard et al. 2011; Stahlschmidt & Brhül 2012), showed us the importance of making contributions to improve and standardise bats surveys, bringing the scientific community together in the efforts of bat conservation.

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(According to conservation biology)

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